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Circular No. 671

March 1943 • Washington, D. C.

UNITED STATES DEPARTMENT OF AGRICULTURE

Sampling Technique for Determining Populations of the Citrus Red Mite and Its Predators

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INTRODUCTION

When a study of the effect of chemical control practices on natural enemies of the citrus red mite (*Paratetranychus citri* McG.) was planned, it was first necessary to develop a method of estimating mite and predator populations on a comparable-unit basis. A method for obtaining an index to the density of field populations of the citrus red mite had been devised and used successfully by Jones and Prendergast. [2] This method was found to be too slow and cumbersome for the proposed study, however, where large numbers of small-unit samples were required. Furthermore, a method was desired by which the samples could be taken and examined immediately. The methods and technique found satisfactory for such a study are herein detailed for the use of future workers along this line of investigation.

DESCRIPTION OF APPARATUS AND TECHNIQUE

The most satisfactory method for rapidly counting mite populations proved to be one whereby the mites and eggs were brushed from the foliage and fruit and concentrated on a small, flat surface for counting.

[1] The writers are indebted to J. K. Holloway for furnishing a diagram of the electrical circuits for installing the collecting apparatus in a car, and for analyzing the data in connection with the field sampling.

[2] JONES, L. S., and PRENDERGAST, D. T. METHOD OF OBTAINING AN INDEX TO DENSITY OF FIELD POPULATIONS OF CITRUS RED MITE. Jour. Econ. Ent. 30: 934–940, illus. 1937.

The apparatus which accomplishes this (fig. 1) uses two ¾-inch cylindrical, or spiral, brushes which are revolved at high speed in opposite directions by an electric motor. The motor also operates a small turntable mounted beneath the brushes on which is placed a 5-inch glass disk. The motor is operated by a 6- or 12-volt storage battery, and should have sufficient speed and power to revolve the brushes at a rate of 1,400 to 1,600 r.p.m. without any appreciable slowing down when the leaf is inserted between them. A pair of

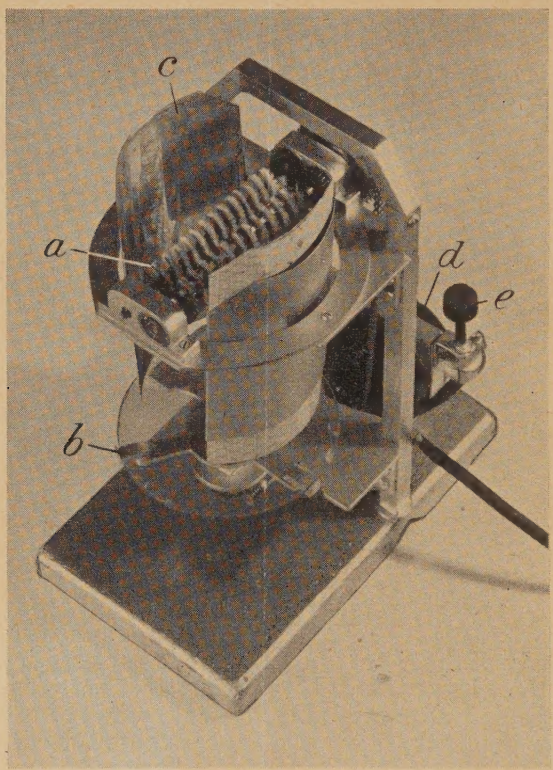

FIGURE 1.—Apparatus for collecting citrus red mites: *a*, Cylindrical brushes; *b* turntable holding a glass disk; *c*, metal slides; *d*, electric motor; *e*, switch.

curved metal slides encloses the revolving brushes and delimits the area on the glass disk which will be occupied by the mites and eggs as they are thrown downward from the brushes. This disk is thinly coated with an adhesive, such as newly applied varnish or other resinous material. Varnish proved to be the most satisfactory, since it was more easily applied than other materials, and the eggs and smaller mites were more readily visible when this adhesive was used. [3]

The operator of the machine inserts the leaf containing the mites and eggs between the brushes (fig. 2) and then withdraws it, first one and then the other end of the leaf being brushed. The mites

[3] Further information about details of the machine or the parts commercially available may be obtained by addressing the Division of Foreign Parasite Introduction, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.

and eggs are thus removed from the leaves and deposited on the varnish-covered disk. As the disk on the turntable is revolved at a constant rate of speed, the distribution of mites and eggs is relatively uniform over the surface. After the mites have been deposited on the disk, the data pertaining to the collection are recorded on the back with a wax pencil, and the plate is then placed in the holding rack (fig. 3).

This rack consists of a metal cylinder having a 1-inch slot on one side. The varnished disks are held apart by inserting metal rings between them. If the collections are to be held for some time, this

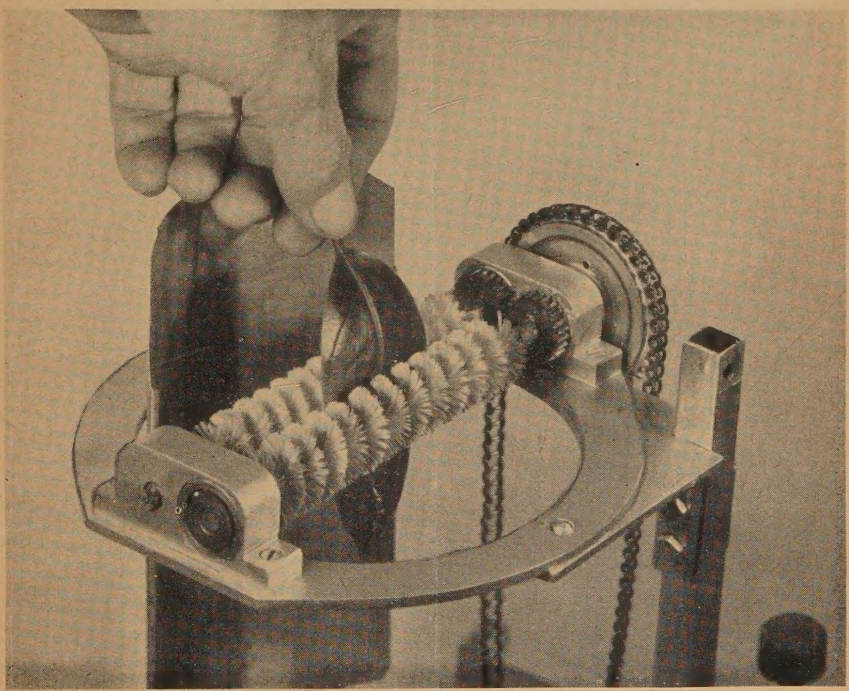

FIGURE 2.—Citrus leaf being brushed in red mite-collecting apparatus. First one and then the other end of the leaf is brushed, the mites and eggs being thrown downward to an adhesive-covered glass disk.

rack can be placed in a refrigerator and the most suitable temperature maintained.

The collecting machine may be operated by a 6- or 12-volt motor, depending on the type of motor and other facilities available. Small 6-volt marine motors with adequate speed and power may now be obtained, and this is probably the most satisfactory arrangement. Diagrams for the installation of both 6- and 12-volt circuits are presented in figure 4. Figure 4, *A*, shows how a supplementary 6-volt battery may be connected in parallel with the car battery for charging, and when this is thrown in series by means of a conveniently mounted switch a 12-volt circuit is obtained at the outlet for operation of the motor. In figure 4, *B*, a 6-volt electrical circuit is shown in which the regular car battery is used to operate a 6-volt motor. In both these installations the car generator is used to keep the batteries charged.

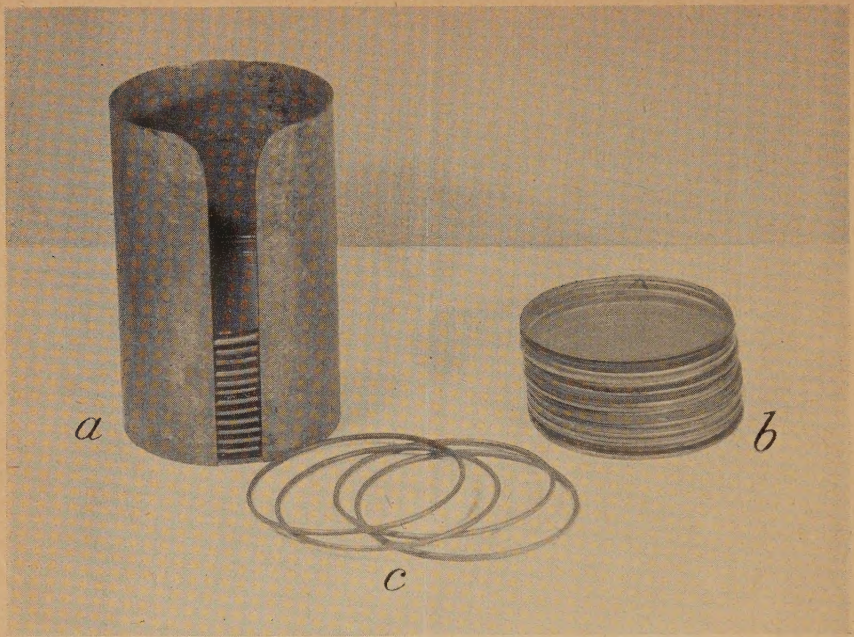

FIGURE 3.—Metal holding rack containing glass disks: *a*, Rack which holds 30 glass disks separated by wire rings; *b*, method of stacking disks in rack; *c*, rings which are inserted between glass disks to keep them from touching.

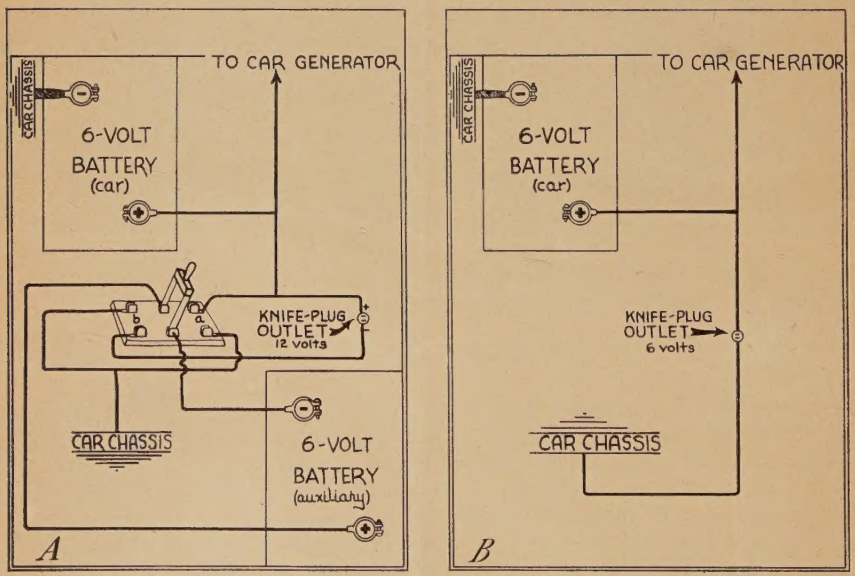

FIGURE 4.—Diagram of the electrical circuits for installing the mite-collecting apparatus in a car: *A*, For use with 12-volt motor; *B*, for 6-volt motor. The cord running from the apparatus is plugged in at an outlet in the car's wiring system.

To count the mites and eggs in a given collection the black count card, or mask (fig. 5), is used. This card contains transparent areas representing approximately 25 percent of the total area covered by the sample. The card is inserted under the glass disk, and the mites and eggs directly above the transparent areas are counted. Illumination provided both above and beneath the count card causes the mites and eggs directly above the areas to become more clearly visible. In addition, when viewed by transmitted light normal eggs can easily be distinguished from eggs in which the contents have become dry. The resulting count represents approximately 25 percent of the total num-

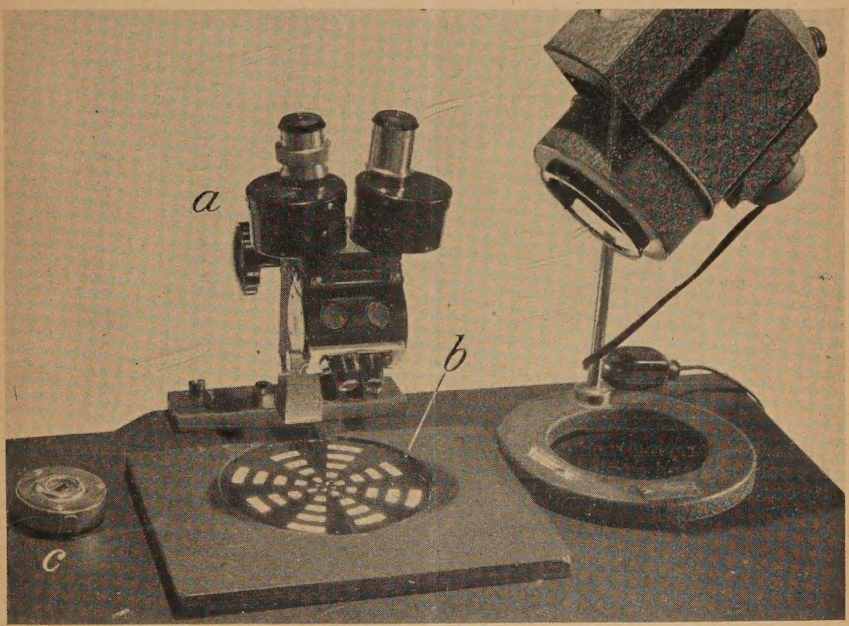

FIGURE 5.—Equipment used in making citrus red mite and egg counts: *a*, Binocular microscope; *b*, count card and varnish-covered glass disk; *c*, hand tally. Substage illumination is provided by a 25-watt light globe mounted in a box attached to the under surface of the table.

ber of mites and eggs on the disks. If the numbers present in one-fourth the sample are not sufficient for an adequate count, the entire disk may be examined under a low-power binocular microscope with little additional work. Extremely low mite populations may be counted in this way.

After the counts have been completed, the disks are placed in a wire-screen rack and submerged in a strong solution of trisodiumphosphate, where they are left until perfectly clean. This is accomplished in a few minutes when a hot solution is used, although just as satisfactory results may be obtained with a cold solution if the disks are left in for a longer time. When all the varnish has been removed the disks are thoroughly rinsed in hot water and then placed in a wooden rack to dry for use again.

When field collections for the determination of mite populations are being made, leaves are removed at random from the trees, placed in ice-cream cartons contained in a carrying rack (fig. 6), and brought to the car for brushing. Fifteen disks are coated with varnish and placed in a wire-screen rack. As soon as the last disk has been treated the first one is ready to receive the mites and eggs brushed from the first 12-leaf sample. As each disk is used it is placed in a metal carrying rack (fig. 3 *a*), being separated from the other disks by one of the metal rings. The time required for varnishing the disks and brushing the leaves is approximately equal to the time consumed in picking the leaves from the trees, or about 20 minutes for 15 average 12-leaf samples.

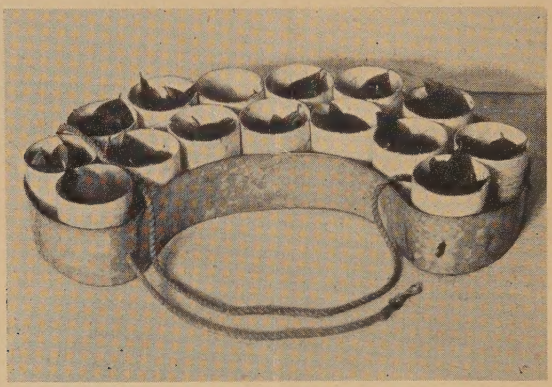

FIGURE 6.—Carrying rack containing ice-cream cartons in which the leaf samples are collected and carried to the car for brushing. The rack is held at the waist by one cord while the other loops over the shoulders and neck.

ADEQUACY OF THE COUNT CARD

The efficiency of the collecting apparatus is determined by the consistency with which a given percentage of the mites and eggs occurring on the leaves or fruit can be accounted for on the disks after they are brushed off. To test this factor, citrus red mites and eggs were carefully counted on the leaves before the brushing, and on the varnish-covered disks afterward. The percentages lost in the process were then calculated.

The ordinary procedure for counting the mites or eggs on the disks is to enumerate only those directly above the illuminated areas, or approximately 25 percent of those on the entire disk. To test the adequacy of this count, 18 samples of infested leaves were brushed and 2 series of counts taken of the eggs present on the disks, one in which the entire area was inspected and the other in which the count card was used and only 25 percent of the area examined. The results of these counts are summarized in table 1. In this table, as in all subsequent tables, the mean percentages with their standard errors were determined from a series of individual percentages.

TABLE 1.—*Recovery of citrus red mite eggs on glass disks after being brushed from citrus foliage, as determined by counts made on the entire disk, and on one-fourth of the disk*

Type of sample	Samples in test	Eggs on each unit before the brushing	Eggs recovered on disk	
			Entire area	One-fourth of area
	Number	*Number*	*Percent*	*Percent*
Single leaf	9	146.0±10.6	80.2±2.3	81.3±2.7
12-leaf sample	9	308.9±38.2	82.9±2.3	83.6±1.9
Weighted mean			82.0±1.6	82.9±1.6

Before the brushing there was an average of 227.5 eggs per unit in a series of 18 samples. When the entire disk was examined 82.0±1.6 percent of the mites originally occurring on the leaves were accounted for, as compared with 82.9±1.6 percent when one-fourth the area was examined and the resulting count multiplied by 4. It is obvious that the use of the count card had little influence on the accuracy of the result, and the counting time was decreased to about one-fourth of that required when the entire disk was examined.

Although the brush-type collecting apparatus has been used exclusively for estimating populations on citrus foliage, an occasional observation indicated that this method might also be employed in making counts on citrus fruit as well. The mites and eggs may be brushed from the fruit by placing the thumb and middle finger on the ends of the fruit and then rotating it over the brushes with the index and third fingers.

ESTIMATING EGG POPULATIONS

To test the efficiency of this method of estimating populations, female mites were first allowed to oviposit on leaves and fruit. The number of eggs deposited was determined before the brushing, and afterward similar counts were made on the glass disks. The leaves and fruit were then examined to determine whether any eggs remained. The results of counts taken of 18 leaf samples and 9 fruit samples are summarized in table 2.

TABLE 2.—*Recovery of eggs of the citrus red mite on glass disks after they were brushed from orange foliage and fruit*

Type of sample	Samples in each test	Eggs per sample before the brushing	Eggs deposited on disks by the brushing	
	Number	*Number*	*Number*	*Percent*
Foliage	18	[1] 227.5±27.5	186.6±23.2	82.0±1.6
Fruit	9	203.9±32.0	167.2±25.4	82.0±1.3
Weighted mean				82.0±1.2

[1] These are the same leaf samples shown in table 1.

An average of 82.0±1.6 percent of the eggs on the foliage samples were recovered on the glass disks for counting, while 82.0±1.3 percent of those on the fruit samples were recovered. To compare populations of mites and eggs between plots, the numbers obtained by using the count card were compared directly. Because of the high and somewhat variable individual percentages recovered, the populations on the glass disks were not multiplied by a factor to correct for the percentage lost in the brushing.

ESTIMATING MITE POPULATIONS

To determine the percentages of mites which could be recovered on the glass disks, the same procedure was followed as was used for egg counts, except that it was necessary to do the counting in a cold room so that the mites would be fairly inactive. Only obviously live mites were counted, all doubtful ones being removed from the leaves or fruit before the brushing. The leaves or fruit were brushed and the mites which were thus deposited on the disks were counted immediately, only the live individuals being enumerated as before. The results of these counts are presented in table 3.

TABLE 3.—*Recovery of citrus red mites on glass disks after they were brushed from orange foliage and fruit*

Type of sample	Samples in each test	Mites per sample before the brushing	Mites deposited on disks by the brushing	
	Number	*Number*	*Number*	*Percent*
Foliage	13	120.5±5.6	106.4±4.7	88.3±1.3
Fruit	15	50.0	43.0±1.1	86.0±2.2
Weighted mean				87.6±1.6

An average of 87.6±1.6 percent of the mites were brushed from the citrus foliage and fruit, as compared with a recovery of 82.0±1.2 percent of the eggs as determined in a previous test (table 2). Most of the mites were in perfect condition, although an occasional one was slightly crushed. These could be easily distinguished from dead mites which had become dry on leaves in the field before the samples had been collected.

THE EFFECT OF THE COLLECTING METHOD ON SAMPLE VARIABILITY

Since the object of the brushing method is to estimate the populations of eggs and mites occurring on field samples of citrus foliage and fruit, a satisfactory collecting technique should be used to assure a consistent percentage of the mites and eggs in the sample. The sample estimate will then have approximately the same relative variability as the sample itself. This variability can be measured by the standard error, and in table 4 the numbers of mites and eggs with their standard errors are given for a series of leaf and fruit samples.

TABLE 4.—*The ratio of the standard error to the mean number of mites and eggs per sample before and after brushing for a series of leaf and fruit samples*

Type of sample	Stage of citrus red mite	Mean individuals per sample			
		On samples before the brushing	Error divided by the mean	On disks after the brushing	Error divided by the mean
		Number	*Percent*	*Number*	*Percent*
Foliage	Eggs	146.0±10.6	7.3	117.1± 8.7	7.4
Do	do	308.9±38.2	12.4	256.0±31.7	12.4
Do	Mites	120.5± 5.6	4.6	106.4± 4.7	4.4
Fruit	Eggs	203.9±32.0	15.7	167.2±25.4	15.2
Do	Mites	50.0		43.0± 1.1	2.5

As shown by the ratio of the standard error to the mean number of mites and eggs per sample before and after the brushing, the variability of the sample was not greatly affected by the brushing technique.

THE EFFECT OF TEMPERATURE AND HUMIDITY ON MITES BEING HELD FOR COUNTING

To determine whether a method could be used to keep the mites in their original condition for approximately 1 week, or until the counts could be completed, tests were conducted in which disks containing mites were placed under various conditions of temperature and humidity, such as (1) under room temperature and humidity, (2) in a humidifier containing moist excelsior and phenol crystals, (3) in a room at 55° F., (4) in an electric refrigerator at approximately 40° F., and (5) first at room temperature and later in a humidifier. Before subjecting the mites to the various conditions, the plump ones, which were alone considered in the tests, were marked by a circle made beneath them on the under surface of the disk with a wax pencil. The results of these tests are shown in table 5.

TABLE 5.—*The effect of various conditions of temperature and humidity on citrus red mites being held on the glass disks for counting*

Test No.	Treatment of mites—disk held—	Total number of mites	Condition of mites after week of treatment	
			Flat	Plump
		Number	*Percent*	*Percent*
1	At room temperature and humidity	43	88	12
2	In humidifier	33	0	100
3	At room temperature of 55° F	37	43	57
4	In refrigerator at approximately 40° F	54	15	85
5	In room, then in humidifier	39	13	87

To demonstrate that dead, dry mites would assume a live appearance when subjected to high humidities, the disk used in the first test containing mites, 88 percent of which were flat, was placed in the humidifier and held for several days. At the end of this time only 13 percent were flat, the others having become medium plump or plump. It is obvious that no satisfactory method has been devised for holding the mites in their original condition for a period of 1 week or over.

When placed in the refrigerator and examined within 3 or 4 days, however, no difficulty was experienced in differentiating mites which were alive at the time of making the collections from those which were dead at that time.

ESTIMATING FIELD POPULATIONS OF MITES AND PREDATORS

To estimate field populations of mites and predators, 12 leaves were removed from each of 10 to 15 trees in each plot or grove. After removal from the tree, each leaf was carefully examined for the presence of predators and the number of these was recorded. The leaf was then placed in an ice cream carton, and when the entire sample had been collected the leaves were brought to the car for brushing.

Single-leaf units are not practicable, since a varnished disk has to be provided for each leaf being sampled. It was demonstrated, however, that not only a single leaf but also either surface of a leaf could be sampled by this method. If it was desired to brush the mites and eggs from one surface of the leaf, the opposite one was covered with a piece of paper and the regular brushing technique employed.

When a study of comparative red mite populations under field conditions is being conducted, the error due to sampling would be influenced by the variation of leaf and tree populations. To measure these sources of variation, population estimates on a leaf and tree basis were made in 2 citrus groves. There are, in general, 2 main pest-control programs practiced, 1 being fumigation with hydrocyanic acid gas and the other the use of an oil spray. Population counts were made in a fumigated grove and in an oil-sprayed grove. In each grove 12 trees were designated as count trees, and a sample of 12 leaves was taken at random from each tree. The red mite population on each leaf was brushed to an individual glass disk so that records would be available for unit-leaf samples.

An analysis of the sources of variation showed that there was a highly significant variation between trees in the oil-sprayed grove, whereas in the fumigated grove the within-tree and the between-tree variations were about equal. In the oil-sprayed grove the mean number of mites per leaf was 9.56 ± 0.76 and in the fumigated grove the mean was 5.96 ± 0.62. In percent, the relative standard error of the mean was slightly higher in the fumigated grove, but it did not exceed 11 percent in either grove. Under the conditions encountered there would be very little reduction in the sampling error by taking more leaves for a tree sample.

Sampling was further tested under grove conditions. A sample of 12 leaves was collected from each of 15 trees and the mites and eggs brushed onto 1 disk, one-fourth of the total disk area being used to determine the population. The trees were successively sampled 7 times with a mean of 18.3 ± 1.5 mites per disk for the subsamples. An analysis showed that the variation between trees was positive and significant as compared with the variation of the subsample means and the variation between subsamples within trees. The variation between subsamples within trees was the lowest source of variation. This indicates only a small amount of disturbance to the mite population resulting from successive collecting, as well as the satisfactory reproducibility of the collecting and brushing technique.

SUMMARY

While the effect of chemical control practices on natural enemies of the citrus red mite was being studied, it was first necessary to develop a method of estimating mite and predator populations on a comparable-unit basis.

A collecting apparatus was developed for brushing the mites and eggs from citrus foliage and fruit and concentrating them on adhesive-covered glass disks for counting. This machine is mounted in an automobile and is driven by an electric motor operated by one or two storage batteries.

In counting the mites and eggs on the glass disk a counting card is used with which 25 percent of the area is illuminated. Only the mites and eggs directly above the transparent illuminated areas are counted.

Efficiency tests of the apparatus and technique indicate that an average of 82.0 percent of the mite eggs occurring on citrus foliage and fruit were recovered on the counting disks. From foliage samples 88.3 percent of the mites were recovered, as compared with 86.0 percent on the fruit.

No satisfactory methods were found for keeping the mites in their original condition for as much as a week, or until counts could conveniently be completed. When they are placed immediately in an electric refrigerator and examined within 3 or 4 days, however, no difficulty was experienced in differentiating mites which were alive at the time of taking the collections from those which were dead.

Predators were counted in the field on the same leaves later brushed for the determination of citrus red mite populations. A single-leaf sample was not practicable, since a varnished disk must be provided for each leaf being sampled.

In the analysis of single-leaf samples taken from a number of trees it was determined that under the conditions encountered there would be very little reduction in the sampling error by taking more than 12 leaves per tree as a sample, but if greater accuracy is desired, the time can be used more advantageously by increasing the number of tree replicates. The variation between subsamples within trees was the lowest source of variation, indicating only a small disturbance to the mite population resulting from successive collecting, as well as the satisfactory reproducibility of the collecting and brushing technique.

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